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# Spontaneous Recovery of Cholinesterases after Organophosphate Intoxication: Effect of Environmental Temperature

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Many compounds are more toxic in the cold environment (Berti and Cima 1955; Harri 1976) and the rate of drug metabolism reactions may change due to changes in ambient temperature (Inscoe and Axelrod 1960). Influence of temperature on the toxicity of organophosphates (OPs) and on the inhibition of cholinesterases (ChEs) is fragmentary and mainly concerned with whole blood ChE and body temperature measurements (Meeter and Wolthuis 1968; Ahdaya et al. 1976; Chattopadhyay et al. 1982). We have found differences in the toxicity of OPs and in the inhibition of ChEs in tissues when subjecting experimental animals to cold (Ryhänen et al. 1987). Now we describe the effect of environmental temperature on the spontaneous recovery of acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) in tissues of rats and mice after exposure to di-isopropylphosphofluoridate (DFP).

## MATERIALS AND METHODS

DFP, 4,4-dithiopyridine and propionylthiocholine iodide were obtained from Sigma Chemical Co. (St. Louis, USA). Acetylthiocholine iodide was from Fluka AG (Buchs, Switzerland). Dithio(bis)nitrobenzoic acid was purchased from Koch-Light Laboratories (Colnbrook, England). The specific BuChE inhibitor Astra 1397 (10-(α-diethylaminopropionyl)-phenothiazine-HCl) was a kind gift from Astra (Södertälje, Sweden).

Male rats (BNxF344F1, 190-230 g) and male mice (Han: NMRI, 30-40 g) at 8 weeks of age from the Laboratory Animal Center of University of Kuopio were used. Animals were under light-dark cycle of 14:10 hours in macrolon cages. Standard pelleted chow (Hankkija Oy, Finland) and water were provided ad libitum. The experiments were always started between 9 and 11 a.m. Groups of five animals were exposed to 20±1 °C or 5±1 C for thirty minutes before injection of DFP (i.p.) in olive oil. Controls received the vehicle only. The dose of DFP was 0.5xLD50 for respective temperature and

species (3.7 and 2.4 mg/kg for rats, 4.9 and 3.2 mg/kg for mice at 20 and 5 °C, respectively).

After 1 to 48 hours, the animals were decapitated, and whole blood was collected to heparinized tubes. Washed tissues were homogenized to cold buffer (50 mM sodium phosphate, pH 7.7). Blood was diluted with 0.1 % Triton X-100, and the ChE activities were measured according to Augustinsson et al. (1978). Homogenates were extracted with 0.2 % Triton X-100, and activities were recorded by the method of Puhakainen et al. (1980) using propionylthiocholine as substrate (acetylthiocholine for brain AChE) and Astra 1397 for inhibiting the BuChE activity. Proteins were determined according to Bensadoun and Weinstein (1976). Rectal temperatures were measured from rats given DFP at 20 and at 5 °C with an Ellab TE3 thermometer (Elektrolaboratoriet, Denmark). We used Students t-test and analysis of variance (ANOVA) in the statistical treatment of the results. Pearson correlations were also calculated.

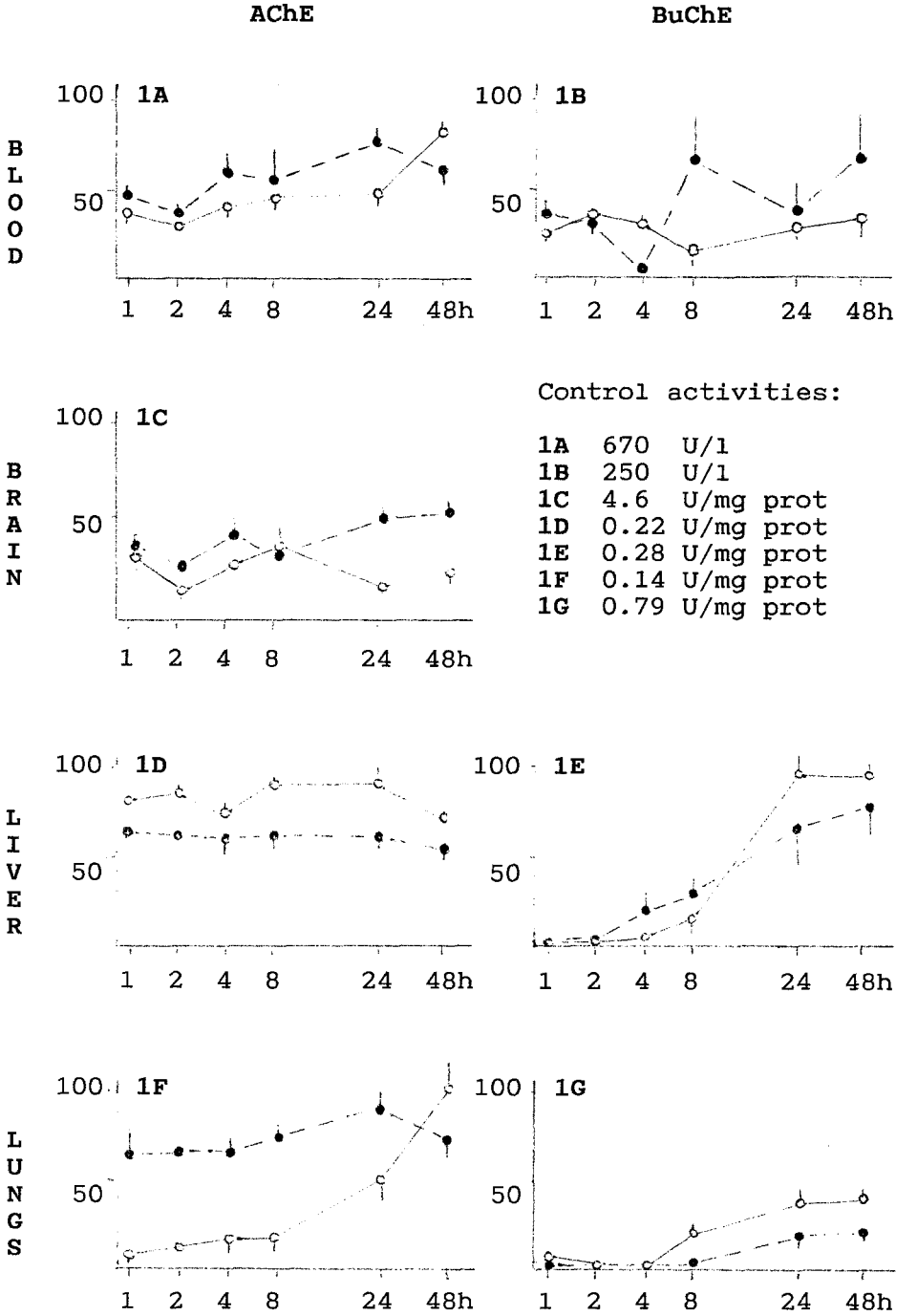
## RESULTS AND DISCUSSION

After DFP-administration, the animals showed involuntary defecation, urination and tremor, which are typical muscarinic and nicotinic symptoms due to excess of acetylcholine. These symptoms disappeared after 4-8 hours.

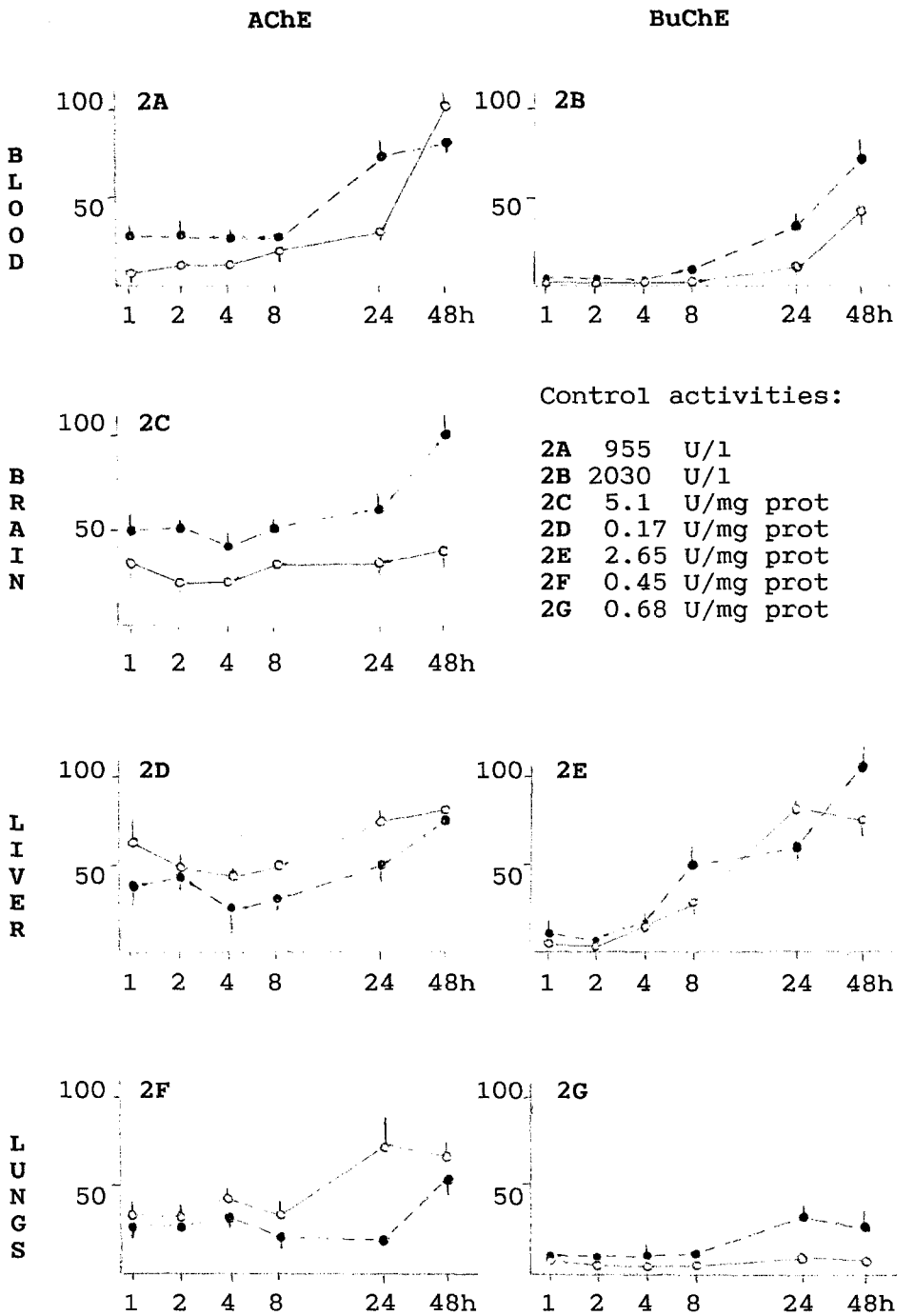
Table 1. Effect of DFP on the rectal temperature of rats exposed to 20 °C and 5 °C

| Time (h) relative to DFP-injection | Intraperitoneal dose of DFP (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | at 20 °C | | | at 5 °C | | |
| | 0 | 2 | 4 | 0 | 2 | 4 |
| 0 | 38.5 ± 0.4 | 38.5 ± 0.1 | 38.5 ± 0.1 | 38.5 ± 0.3 | 38.4 ± 0.3 | 38.3 ± 0.2 |
| 1/2 | 38.5 ± 0.4 | 36.0[a] ± 0.2 | 35.6[a] ± 0.5 | 38.2 ± 0.3 | 36.0[a] ± 0.5 | 35.6[a] ± 0.9 |
| 1 | 38.6 ± 0.4 | 35.6[a] ± 0.4 | 34.6[a] ± 0.8 | 38.3 ± 0.3 | 36.7[ab] ± 0.3 | 32.9[a] ± 1.7 |
| 2 | 38.5 ± 0.2 | 35.7[a] ± 0.4 | 33.3[a] ± 0.6 | 38.0 ± 0.6 | 36.4[ab] ± 0.5 | 32.5[a] ± 1.7 |
| 4 | 38.4 ± 0.3 | 35.5[a] ± 0.5 | 32.6[a] ± 0.9 | 38.1 ± 0.4 | 36.5[ab] ± 0.7 | 32.1[a] ± 2.1 |
| 24 | 38.4 ± 0.4 | 38.0 ± 0.3 | 36.0[a] ± 0.7 | 37.3 ± 0.3 | 37.3 ± 0.3 | 36.3[a] ± 1.0 |

Values are expressed as °C (mean + SD, n=5). 0 h = after 30 minutes at 20 or 5 °C. Indices denote statistically significant ($p < 0.05$) difference to control of same group (a) and between groups (b).




Figures 1A-1G. Recovery of rat tissue AChE and BuChE after a dose of DFP (0.5xLD50, i.p.) at 20 °C (o--o) and at 5 °C (●--●). Values are means from five animals and expressed as percent of control activity (values shown separately). SD is shown by vertical bar when greater than the size of symbol. Control values between 20 °C and 5 °C did not differ significantly.




Figures 2A-2G. Recovery of mouse tissue AChE and BuChE after a dose of DFP (0.5xLD50, i.p.) at 20 °C (o—o) and at 5 °C (●--●). Values are means from five animals and expressed as percent of control activity (values shown separately). SD is shown by vertical bar when greater than the size of symbol. Control values between 20 °C and 5 °C did not differ significantly.

Table 1 shows that DFP produced dose-dependent hypothermia. It returned to near-normal values after 24 h. At 24 h a significant decrease ($p < 0.01$) in the rectal temperature of control animals exposed to 5 °C was seen. The cold exposure protected the animals from hypothermia at dose of 2 mg/kg, but had no effect at the higher dose. Lowest rectal temperatures were recorded during 1-4 h after dosing.

Figures 1A and 1B show that the inhibition of rat blood ChEs was maximal at 2 h after injection of DFP and the recovery was virtually minimal thereafter; only blood AChE was elevated to 70 % of control value during 48 hours. In mice, the return of enzymatic activities was significant (Figs 2A, 2B). Furthermore, the recovery of blood BuChE was about 2-fold faster in the cold (ANOVA, $p < 0.001$). Rat brain AChE returned only slightly, but in mice the values at 48 h were about 40 % (20 °C) and 90 % (5 °C) of the controls (Figs 1C, 2C).

The hepatic AChE could be inhibited only to 40-60 % of control activity from which it did not recover during 48 h (Figs 1D, 2D). On the other hand, BuChE rose rapidly to about 70 % of control activity in 24 h (Figs 1E, 2E). In lungs, AChE recovered significantly only at 20 °C (Figs 1F, 2F) during the first 24 hours. BuChE showed, however, only marginal regain of activity (Figs 1G, 2G). Rats and mice responded differently to cold exposure. Despite the lower absolute dose, pulmonary AChE was constantly lower in mice at 5 °C than at 20 °C ($p < 0.05$). In rats pulmonary BuChE behaved similarly.

Vandekar et al. (1971) have reported that cholinergic symptoms begin when blood and brain ChE activities fall below 50 %. In our studies, the major signs disappeared after 4-8 h when blood ChEs were about 25 % (mice) to 40 % (rats) of control values and brain AChE had reached the value of 30-40 %.

The rectal temperature of rats decreased showing that the most severe hypothermia occurred during the first four hours after DFP-injection, as also described by Meeter et al. (1971). Heat loss may be due to vasodilatation (Meeter and Wolthuis 1968) and inhibition of shivering thermogenesis (Harri et al. 1984). Temperature lowering coincided with the greatest inhibition of ChEs and paralleled the recovery of blood ChE activity. This finding confirms the results of Chattopadhyay et al. (1982). The brain AChE activity, however, responded differently, which might explain dissimilar recovery of neuronal functions in different regions of the brain. It is known that the reactivation of brain AChE in guinea-pigs is very slow (Aldridge and Reiner 1972).

The present results showed that the spontaneous recovery of ChEs after DFP-intoxication differed greatly in

different tissues. Therefore the measurement of whole blood ChE does not necessarily reflect the severity of the inhibition of brain AChE ($r = 0.150$, $p > 0.2$, $n = 29$) adequately. BuChE rose rapidly in the liver regardless of experimental temperature and it correlated poorly to other ChEs ($r < 0.153$, $p > 0.1$, $n = 56$).

Rats appear to have a poor ability to recover AChE; only blood and lung AChE approached control values at 20 °C but not at 5 °C. In mice tissues, the rise of ChE activities tended to be more effective than in rats. Blood BuChE recovered almost twice as fast in the cold than at 20 °C. It is known that mice metabolize parathion faster than rats (Guthrie et al. 1974). There are great differences in the levels of DFP-detoxicating enzymes between species and also between various organs of a species (Chemnitius et al. 1983). Differential inhibition and recovery was seen in lung ChEs between rats and mice, which could partly be due to dissimilar changes in blood circulation when exposing animals to cold. All ChEs except lung AChE correlated quite well to blood AChE and BuChE ($r > 0.713$, $p < 0.01$, $n = 56$).

We have shown that after DFP-intoxication (dose levels of 2 to 16 mg/kg) only brain and lung AChE activities correlate statistically significantly to blood AChE in this rat strain. In mice, blood ChEs correlated to the degree of ChE inhibition in brain and other tissues (Ryhänen et al. 1987). It is concluded that measurement of whole blood ChE might not always adequately describe the inhibition of ChEs in tissues. Cold exposure affects the recovery of blood BuChE in mice and ChEs in lungs.

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